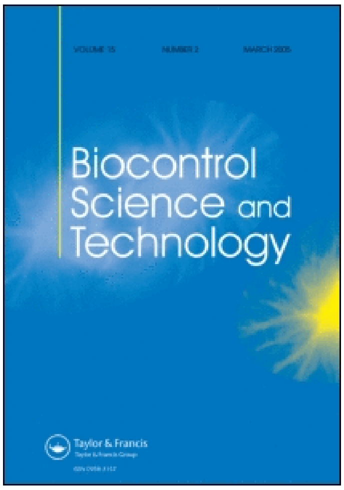

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SHORT COMMUNICATION

Occurrence of invertebrate-pathogenic fungi in a Cerrado ecosystem in Central Brazil

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Occurrence of invertebrate-pathogenic fungi in a protected area of the Cerrado region of Brazil is reported. Fungi were baited with triatomines, ticks, aquatic snails or mosquito larvae from substrates or collected from infected insects. The findings underscore the importance to preserve these fungi and to investigate their potential for vector control.

Keywords: biological control; conservation; Entomophthorales; Hypocreales; isolation; vector

The Cerrado is the largest savanna in South America and comprises nearly 2 million km^2 located mainly in Central Brazil. Although this region is considered to be one of the world's top biodiversity hotspots, this ecosystem is threatened by large single-crop plantations, cattle production, criminal fires, and other human activities. Only about 20% of the original area of the Cerrado remains without human interference, and less than 3% of its area has been protected by law (Mittermeier et al. 2005). The region was originally characterized by extensive savanna and forest formations and has a hot, semi-humid climate with a dry winter season from May through September or October (Klink and Machado 2005). The overall reduction of biodiversity of its plants and animals and the implicit, undocumented extinction of possibly useful microorganisms are irreversible. Little information exists about the occurrence and biocontrol potential of entomopathogenic fungi or other beneficial microorganisms present in this biome (Shimazu, Alves, and Kishino 1994; Luz, Rocha, and Nery 2004; Monnerat et al. 2004). Intensified collecting efforts together with monitoring of mycoses affecting arthropods and their dynamics are necessary in order to ensure a proper *in situ* preservation of pathogenic fungi and of their specific benefits for integrated pest control. We report the occurrence of invertebrate-pathogenic fungi in a protected area of tropical gallery forest area in the Cerrado in Central Brazil.

Dry soil or samples of water from small ponds and marginal mud slurries were collected during the dry season in 2006 in a tropical primary gallery forest located in

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the Santa Branca Farm, ca. 40 km NE of Goiânia in Central Brazil (-16° 23′ 41″ latitude and -49° 04′ 47″ longitude, WGS 84). At randomly selected locations that were protected against continuous sunlight by vegetation, 25 g substrate were removed to a depth to 2–3 cm, or 1500 mL water were taken from the water surface, transferred to plastic bags and stored in a polystyrene cooler at 20°C.

In the laboratory, five invertebrate species of importance to human or animal health were used to bait fungi from these samples for subsequent isolation into pure cultures. Three third-instar nymphs (N3) of the laboratory-reared triatomine *Rhodnius neglectus* Lent or three engorged *Boophilus microplus* (Canestrini) female ticks collected on acaricide-free cattle were exposed in Petri dishes (90 mm diameter) to 3 g of each homogenized soil or mud slurry (that was drained previously through sterile gauze) and incubated in a humid chamber ($40 \times 37 \times 27$ cm) with a saturated solution of K_2SO_4 at $\geq$98% relative humidity (RH) (Winston and Bates 1960). One individual of the aquatic snail *Biomphalaria glabrata* (Say) or 10 second-instar larvae (L2) each of the mosquitoes *Aedes aegypti* (L.) and *Culex quinquefasciatus* Say, all reared in the laboratory, were exposed to residua of 1000 mL samples of water after a 24-h sedimentation, or to suspended soils or slurries (3 g in 15 mL sterile tap water) filtered previously through sterile gauze (5 mL collected residuum, suspended soil or slurry in 45 mL sterile tap water). All exposed invertebrate bait species were incubated at 25°C with a photophase of 12 h. Mosquito larvae were fed with small amounts of ground pellets of cat food (Black Jack, Alisul Alimentos S.A., São Leopoldo, Rio Grande do Sul, Brazil), and snails with small cubes made of 20 g oatmeal, 20 g calcium carbonate, 20 g agar, 20 g milk powder, 500 mL water (Vinaud, Mendes, and Bezerra 2001). Triatomines and ticks were not fed. Mortality was monitored daily for 15 days. Dead triatomines and ticks were dipped in 93% alcohol followed by 2.5% sodium hypochlorite for 3 min, washed three times for one min in sterile water and then transferred to a Petri dish (90×15 mm). Cadavers were incubated on filter paper in a moist chamber for 10 days at 25°C, and fungal development on the cadavers was evaluated daily. Dead larvae and snails were transferred directly onto an antibacterial agar medium (AM: 18 g agar, 0.5 g chloramphenicol, 10 mg crystal violet, 1000 mL water, pH 5) to facilitate initial fungal development on cadavers; fungi emerging on cadavers were inoculated onto complete medium (CM: 0.001 g $FeSO_4$, 0.5 g KCl, 1.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 6 g $NaNO_3$, 0.001 g $ZnSO_4$, 1.5 g hydrolysed caseine, 0.5 g yeast extract, 10 g glucose, 2 g peptone, 20 g agar and 1000 mL distilled water, pH 7) amended with chloramphenicol (0.5 g/L medium). All fungi were identified morphologically (Humber 1997) and stored in the collection of entomopathogenic fungi at IPTSP (Instituto de Patologia Tropical e Saúde Pública).

Dead invertebrates infected by fungi were collected in this same area during the rainy season in 2006/2007. Fungi were identified morphologically, and the diseased field-collected insects were stored at IPTSP.

A total of 75 samples were collected: 45 of soil, 15 of slurry and 15 of water. Of 68 fungal isolates obtained from mycotized invertebrate baits, 73.5% were derived from soils, 22.1% from slurries and 4.4% from water samples (Table 1). Among these isolates, 76.5% were baited from *R. neglectus*, 10.3% from *B. microplus*, and 4.4% from each *B. glabrata*, *A. aegypti* and *C. quinquefasciatus* (Table 1). The isolates were identified as *Metarhizium anisopliae* (Metsch.) Sorokin (22 isolates), *Paecilomyces lilacinus* (Thom) Samson (13), *Pochonia chlamydosporia* (Goddard) Zare and W. Gams

Table 1. Fungi isolated from substrates collected in a tropical gallery forest, Goiás, Brazil with different invertebrate host baits and their respective quantity and IP codifications of the fungal collection at IPTSP, UFG.

Fungus	Host bait: *Rhodnius neglectus*	*Aedes aegypti*	*Culex quinquefasciatus*	*Boophilus microplus*	*Biomphalaria glabrata*
Aspergillus sp.	–	1 (IP 284, a)	1 (IP 285, a)	–	–
Beauveria sp.	2 (IP 306; IP 308, b)	1 (IP 305, b)	–	–	1 (IP 307, b)
Cladosporium cladosporioides	–	1 (IP 287, b)	–	–	–
Evlachovaea sp.	1 (IP 304, b)	–	–	–	–
Fusarium sp.	4 (IP 298, b; IP 297; IP 299; IP 300, c)	–	–	2 (IP 295; IP 296, c)	–
Gliocladium sp.	4 (IP 290; IP 291, IP 293; IP 294, c)	–	–	–	1 (IP 292, b)
Isaria farinosa	1 (IP 303, b)	–	–	–	–
Lecanicillium psalliotae	1 (IP 301, c)	–	–	–	–
Metarhizium anisopliae	20 (IP 332; IP 333; IP 337 – IP 353, b; IP 336, c)	–	–	2 (IP 334, b; IP 335, c)	–
Paecilomyces lilacinus	10 (IP 319; IP 331, c; IP 323 – IP 330, b)	–	1 (IP 320, a)	2 (IP 321; IP 322, b)	–
Pochonia chlamydosporia	9 (IP 311 – IP 318, b; IP 310, c)	–	–	1 (IP 309, b)	–
Trichoderma sp.	–	–	1 (IP 288, b)	–	1 (IP 289, b)
Total isolates	52	3	3	7	3

Isolated from a: water (3 isolates), b: soil (50 isolates) and c: slurry (15 isolates).

(10), *Fusarium* sp. (6), *Gliocladium* sp. (5), *Beauveria* sp. (4), plus two isolates each of *Aspergillus* sp. and *Trichoderma* sp., and single isolates each of *Cladosporium cladosporioides* (Fresen.) de Vries, *Evlachovaea* sp., *Isaria farinosa* (Holm ex S.F. Gray) Fr and *Lecanicillium psalliotae* (Treschow) Zare and W. Gams.

The field-collected diseased insects presented a substantially different group of fungi from those obtained using bait species. During three visits in the rainy season at the end of October and beginning November an epizootic of a *Batkoa* species was found in this forest with hundreds of small nematoceran mycotized flies (Diptera) (Figure 1) that were attached to the underside of the leaves. Several hemipteran hosts were collected with mycoses identified as bearing both *Aschersonia* sp. and its corresponding teleomorph, *Hypocrella* sp.; a *Fusarium* sp. was found on whiteflies (Aleyrodidae), *Torrubiella* sp. on scale insects (Coccoidea). *Evlachovaea* sp. and *Lecanicillium* sp. attacked other unidentified species of Hemiptera. *Batkoa apiculata*

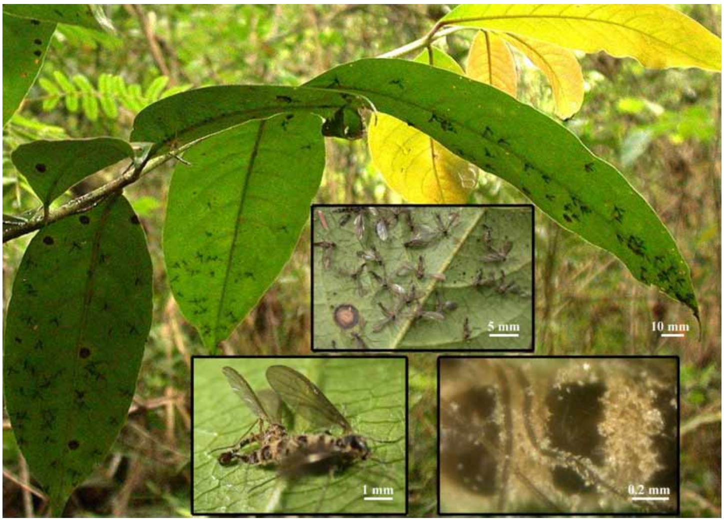

Figure 1. Epizootic of a *Batkoa* species found on small nematoceran flies (Diptera) in a tropical primary gallery forest in Central Brazil during the rainy season in 2006/2007.

and *Beauveria* sp. were detected on a coleopteran, and a *Pandora* sp. was found on a lepidopteran insect. *Cordyceps lloydii* was found on ants (Hymenoptera, Formicidae).

The overall results confirmed the occurrence of invertebrate-pathogenic fungi in this preserved area. It is noteworthy that most fungi, including the nematode pathogens *P. chlamydosporia* and *P. lilacinus*, were isolated using *R. neglectus*, a peridomestic potential vector of *Trypanosoma cruzi* in Central Brazil. This and other triatomines are highly susceptible to fungal infections at high moisture (Luz, Silva, Cordeiro, and Tigano 1998). Various isolates of *Evlachovaea* sp. have been recently isolated in the State of Goiás and other regions of Brazil (Humber, Tanzini, and Alves 2002; Luz, Rocha, and Humber 2003; Humber and Hansen 2006). One of these isolates was found on a dead *Triatoma sordida* and was active also against other triatomine species when tested under laboratory conditions (Luz, Rocha, and Silva 2004). Results of recent morphological and molecular studies on *Evlachovaea*-like isolates collected in Central Brazil suggest that at least two different groups of these little known fungi occur in the Cerrado and that they may be more common than expected (L.F.N. Rocha, P.W. Inglis, R.A. Humber and C. Luz, unpublished manuscript).

Other fungi such as *M. anisopliae* and species of the genera *Aspergillus*, *Beauveria*, *Cladosporium*, *Fusarium*, *Gliocladium*, *Isaria*, *Paecilomyces* and *Trichoderma* detected at Santa Branca are widespread, and great importance is attached to some species of the mentioned genera as pest control agents (Faria and Wright 2007). Despite the presence in the Cerrado of *Fusarium* sp., *P. lilacinus* and *P. chlamydosporia* baited with *B. microplus*, it is the activity of *M. anisopliae* against ticks that has been well recognized and studied (Samish and Rehacek 1999).

Although few fungi were isolated from the snail *B. glabrata*, it is notable that the important entomopathogenic genus *Beauveria* seemed to be active against this major intermediate host of *Schistosoma mansoni* in Latin America and in other regions. Further investigations of the possible activities of entomopathogenic fungi against such molluscan vectors of significant helminth disease organisms seem to be warranted by this result.

P. lilacinus that was shown to have ovicidal activity in *A. aegypti* (Luz et al. 2007) was isolated from *C. quinquefasciatus* larvae. Moreover, baiting fungi from the genera *Aspergillus* and *Beauveria* with both aedine and culicine mosquito larvae underscores the potential of entomopathogenic fungi for biological mosquito control.

In the present study *C. lloydii* and an unidentified species of *Torrubiella* sp. are reported for the first time in Brazil, and *Aschersonia* sp., *L. psalliotae*, and *B. apiculata* for the first time in a Cerrado area, but these last fungi have been found previously in the State of São Paulo (Batista, Leite, Takada, Lamas, and Ramiro 1997); *B. apiculata* and various species of the genus *Pandora* were reported from southern Brazil (Sosa-Gómez and Humber 2002). Moreover, one *Batkoa* sp. isolate was detected in the State of Bahia (Sánchez, Freitas, and Roberts 2001) and *Pandora delphacis* was isolated from homopteran insects in Goiânia, Goiás, in 1984 and 1985 (Humber and Hansen 2006). Various isolates of *Aschersonia* sp. and an *A. aleyrodis* were found on *Trialeurodes citri* (Homoptera) and *Bemisia* sp. in 1994 and 1998 in south eastern and southern Brazil, respectively (Tigano, Faria, and Martins 2002; Humber and Hansen 2006), and finally an epizootic caused by *Aschersonia* cf. *goldiana* from *Bemisia tabaci* (Homoptera) was reported in 1999 by Lourenção, Yuri, and Alves (1999) in the State of São Paulo.

The recovery of notably different fungi obtained either by exposing bait species of invertebrates to soil, water, or other materials recovered from a site as compared to those obtained by field collections of naturally mycosed insects merits some comment. Pathogenic fungi recovered through baiting tend to be the most generalized pathogens that are able to infect a wide range of potential hosts either as primary or facultative pathogens. The generalist species of genera such as *Beauveria*, *Metarhizium*, *Lecanicillium*, *Paecilomyces* and *Isaria* should be expected to be recoverable from mycosed hosts if they are recovered through bait species. Those fungi with much narrower specificities for particular types of hosts (e.g., hosts from single families or superfamilies of an order, especially within the Hemiptera) cannot be recovered from any bait species for which these pathogens are noninfective. The most complete recovery of an entomopathogenic mycobiota from a given ecosystem requires diligent collecting throughout all periods of the year as well as such indirect sampling regimes as baiting with a taxonomically diverse range of potential hosts.

The effective conservation management of remaining areas with elevated biological diversity of invertebrate pathogens could facilitate a migration of virulent species and strains to close-by areas with poorer genetic diversity and, thereby, contribute to a natural control of pests. Prospecting activities of invertebrate-pathogenic fungi and a permanent safe keeping of new isolates in specialized collections will facilitate and assure a better conservation of species that have not been described or evaluated for pest control.

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